B3 Cont'd

subunits, and (iii) the DNA sequences are non-covalently bound to a polycationic polymer on the surface of the substrate.

Please add new claims 38-39 as follows:

B4

38. (New) A substrate with a surface comprising a microarray of DNA sequences of claim 7 or 36, wherein the DNA sequences are distinct gene sequences whose expression levels are specifically related to the differences between test cells relative to control cells.

39. (New) A substrate with a surface comprising a microarray of DNA sequences of claim 34, wherein the DNA sequences are distinct gene sequences whose expression levels are specifically related to the differences between test cells relative to control cells.

II. REMARKS

(a) Status of claims:

Claims 7-37 have been examined and are rejected on various grounds. By virtue of this Amendment, claims 7, 34, and 36 are amended to more clearly point out and distinctly claim the subject matter which Applicants regard as the invention. Support for the amendment can be found throughout the specifications including the parent applications U.S. Serial No. 08/477,809, now U.S. Patent No. 5,807,522. Specifically, the recitation of "at least 50 subunit in length" in these claims is supported by examples and descriptions in the instant application and its parent applications. In particular, support for the length limitation can be found at page 7 lines 27 of U.S. Serial No. 08/477,809, and page 9 lines 5-7 of U.S. Serial No. 08/688,488, now abandoned.

New claims 38-39 are added. Support for the two claims is generally found at page 38 under the embodiment of "Subarray Device and Method" in the priority application U.S. Serial

No. 08/514,875. In addition, page 5 lines 9-12 describe that the genes on the microarray show a significant elevation or reduction in reporter levels, when compared with control levels.

An issue of new matter is not raised by these amendments, and entry thereof is respectfully requested. Upon entry of this Amendment, claims 7-39 are now pending.

(b) Informalities:

Objection to the drawings indicated by the Examiner is acknowledged. Correction will be made where needed before issuance of this application.

Notification to comply with the requirements directed to applications containing nucleotide and/or amino acid sequence is also acknowledged. Submitted herewith is the sequence listing in compliance with 37 CFR §1.821-1.825.

(b) Interview Summary:

At the outset, Applicants express their sincere appreciation to Examiner Ardin Marschel for a constructive interview on October 18, 2000 leading up to this response. Discussed during the interview include the references U.S. Patent Nos. 5,744,305 (by Fodor et al.) and 6,040,193 (by Winkler et al.). The Examiner expressed his view "as to difficulty of Fodor et al. in making polynucleotides on the array at significant length beyond short oligomers." See Examiner Interview Summary Record dated October 18, 2000.

In view of those discussions and the amendments and remarks made herein, the application is believed to be in condition for allowance, and an early Notice of Allowance is respectfully requested.

(c) Non-statutory double patenting rejection

Claims 21-33 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-7 of U.S. Patent No. 5,807,522. Applicants

have submitted herewith an executed Terminal Disclaimer as suggested by the Examiner to overcome this rejection. Applicants respectfully submit that claims 21-33 of the application are now in allowable condition.

(d) Rejection under 35 U.S.C. §102(e)

Claims 7-20 and 34-37 stand rejected under 35 U.S.C. §102 (e) as being allegedly anticipated by Fodor et al. (U.S. Patent No. 5,744,305). Fordor et al. is cited for disclosing high density arrays of polynucleotides. Applicants respectfully traverse. First, Fodor et al. fails to teach each and every element of the claims as originally presented. Second, it fails to provide an enabling disclosure for one skilled in the art to construct arrays of nucleic acids having the required length (i.e. at least 50 subunits).

Fodor et al. teaches a photolithographic method (known as VLSIPS technique) for light-directed synthesis of oligonucleotide arrays. At column 27, lines 35-47, Fodor et al. states that the method "*requires the stepwise attachment of a nucleotide to a substrate-bound growing oligomer. In order to prevent unwanted polymerization of the monomeric nucleotide under the reaction conditions, protection of the 5'-hydroxyl group of the nucleotide is required. After the monomer is coupled to the end of the oligomer, the 5'-hydroxyl protecting group is removed, and another nucleotide is coupled to the chain. This cycle of coupling and deprotecting is continued for each nucleotide in the oligomer sequence.*"

Fodor et al.'s *in situ* method of stepwise coupling and deprotection of monomeric nucleotide has several pronounced limitations. Notably, the coupling step is not one hundred percent efficient, nor is the deprotection step effected by illumination of selected regions using a mask. Consequently, the growing nucleotide polymers are often prematurely truncated; unintended sequences are synthesized within discrete regions; and necessarily, each region on the resulting array is not free of cross-contamination with nucleic acid sequences synthesized in

the other regions on the array, which is a structural characteristic required by the instant invention.

Indeed, Fodór et al. concedes this and other structural distinctions of the claimed microarrays, by acknowledging the intrinsic limitations of its *in situ* synthesis method. For example, column 17, lines 59-67 of Fodor et al. describes:

> **"Another important consideration is the fidelity of synthesis. Deletions are produced by incomplete photodeprotection or incomplete coupling. The coupling yield per cycle in these experiments is typically between 85% and 95%. Implementing the switch matrix by masking is imperfect because of light diffraction, internal reflection, and scattering. Consequently, stowaways (chemical units that should not be on board) arise by unintended illumination of regions that should be dark."**

Calculation based on Fodor et al.'s conceded coupling efficiency illustrates that Fodor et al. would fail to enable one of ordinary skill in the art to synthesize a nucleotide polymer much longer than 20 mers. Nor would Fodor et al. enable a microarray with a homogenous population of polynucleotides immobilized within each discrete region. For instance, assuming the average coupling yield per cycle is 90%, the expected percentage of yield for generating a 25 mer is approximately 8%[1] ($= 0.9^{24}$). Thus, within a synthesis region where a population of 25 mers is to be synthesized, there will be on average 90% unintended sequences. A majority of them contain base deletions and/or truncations. Clearly, Fodor's monomer-by-monomer photoactivating technique as disclosed in the cited patent cannot generate regions of homogenous population of polynucleotides having at least about 50 subunits. Fodor's elaborate synthetic scheme, is limited to relatively short nucleic acid sample, e.g. less than 20 bases. Furthermore, imperfect masking results in unintended illuminations of regions and hence "stoaways" oligonucleotides. Applicants recognized this and other limitations (see the great-grand parent priority application U.S. Serial No. 08/261,388, first sentence at page 3), and devised a novel

[1] This calculation assumes that the first cycle of coupling is complete.

method for generating a high-density array without such structural limitations of the cited Fodor arrays.

Embodied in the instant application are a unique apparatus and method for depositing a vast number of pre-formed polynucleotides onto an array surface. The apparatus comprises a tweezer-like, open-capillary dispenser tip. As described in the instant and the priority applications, the open-capillary dispenser tip facilitates rapid, efficient washing and drying before reloading the tip with a new reagent (see priority applications U.S. Serial Nos. 08/261,388 and 08/477,809 at pages 12 and 18, respectively). Such structure is also less prone to clogging than closed capillaries, unlike the ones disclosed in Winkler et al. This apparatus and method enable the generation of an array immobilized with regions of homogenous polynucleotides in any length to a solid substrate. The resulting arrays (including the arrays covered by claims 21-33) contain homogenous populations of polynucleotides localized in discrete regions that are free of cross-contamination of polynucleotides applied to other regions. Furthermore, because the polynucleotides are pre-formed either naturally or by synthetic means, the array polynucleotides can be substantially longer than oligonucleotides formed by the cited, *in situ* step-wise polymer synthesis method.

Applicants submit that the cited art neither anticipates nor renders the claimed arrays obvious. The cited disclosures fail to enable one skilled in the art to construct an array of polynucleotides having the required length, and/or the required homogenity. Withdrawal of this rejection is respectfully requested. Since the independent claims 7, 34, and 36 are not anticipated over the cited art, neither are the dependent claims that recite additional limitations.

III. CONCLUSION

Applicants respectfully submit that the above amendments and remarks fully respond to the rejection made in the Office Action mailed June 20, 2000. Applicants submit that the claims as amended are in allowable form and condition. If the Examiner believes a telephone interview

would further prosecution of this case, the Examiner is invited to call the undersigned at (650) 463-8100.

It is believed that a sufficient extension fee is included; however, should any fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason, the Assistant Commissioner is authorized to deduct said fees from Arnold White & Durkee Deposit Account No. 01-2508/STFD:009--1/RHO.

Respectfully submitted,

Dated: December 20, 2000

Karen K. Wong (Reg. No. 44,409) for
Glenn W. Rhodes (Reg. No. 31,790)

HOWREY SIMON ARNOLD & WHITE, LLP
P.O. Box 4433
Houston, Texas 77210-4433
(650) 463-8100
Attorneys for Applicants